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> IMPRESSUM

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ROMER Romer Labs®

Spotlights

Volume 12

> EDITORIAL

Originally developed to guarantee NASA's space food quality, HACCP has evolved to be a standard in today's food and feed industry. HACCP has also become a synonym for quality assurance and can even be considered as a brand. For instance, a study in China has revealed that customers are paying a 5% premium for products labeled with a HACCP logo. However, HACCP is a theoretical concept which is only as good as its realization within an industry environment. A practical approach meeting the industries' demands is as important as an integral conception throughout the value chain. Especially in the case of mycotoxins, where multiple possible origins of fungal infection can occur, any prevention strategy for fungal and mycotoxin contamination must be carried out at an integrative level all along the food production chain.
Romer Labs® products and services address the entire agriculture and food value chain from screening at the producing farms to highly sophisticated reference laboratories – therefore Romer Labs® is the ideal partner for your HACCP concept and can help to monitor mycotoxins at all stages of your production chain.

Gerald Gutscher

The use of HACCP (Hazard Analysis and Critical Control Points) systems to guarantee the production of safe food products for all types of consumers has become very popular over the last decades. The HACCP technique is a logical, straightforward control system based on the prevention of problems, in other words, the use of common sense in the management of food safety.[1,2]

Monitoring Mycotoxins at HACCP Control Points

Basically, for the implementation of a functioning HACCP system the following five successive steps are recommended:

- Observing the process/product from beginning to end.
- Identifying potential hazards and determining during which part of the process they may arise.
- Establishing controls and supervising them.
- Keeping written records of everything.
- Ensuring the system continues to work efficiently.

The object of this article is to present adequate options for monitoring control points in a mycotoxin HACCP system. However, first certain concepts must be made clear, especially regarding the characterization of hazardous mycotoxins. Although mycotoxins are chemical compounds that appear as residues in food, they are considered biological hazards rather than of chemical hazards because their presence is a direct consequence of fungal contamination at some point in the system.[3,4]
Cereals and nuts are the most sensitive food products to mycotoxin contamination. Nonetheless, these residues can also be detected in products of animal origin such as milk, meat,

eggs, and plant products such as coffee, wine, dried fruit, etc. The variety of foods that can be contaminated by mycotoxins is as vast as the type of contaminating mycotoxins; therefore, establishing a single model for a mycotoxin control system in foods is no simple task.

Monitoring methods: fungal or mycotoxin analysis?

As previously mentioned, despite being chemical substances mycotoxins are considered biological hazards. This could lead us to believe that the correct methods for monitoring control points are those that detect, quantify, identify and classify fungi. First, we must consider that in many cases the different processes eliminate fungi from the substrate, but as mycotoxins are so stable they remain throughout the food processing chain; in other situations, potential mycotoxigenic fungi might be detected but are not producing toxins because it is either a non-producing strain, the substrate is inadequate, or just because the environmental conditions are not propitious for generating the mycotoxin in question.

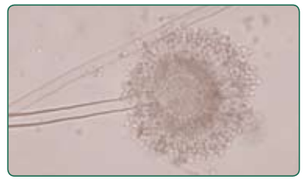
In addition to a possible lack of correlation between the presence of potentially toxigenic fungi and mycotoxins in a particular food product, we must consider the available methods of fungal analysis in foods:

- Classical counting, identification and classification-

characterization methods for detecting microbial contamination. The disadvantage of these methods is the time required to reach a useful answer – in other words, counting and identifying potentially toxigenic strains and their capacity to produce mycotoxins in the studied substrate. Obtaining the end results for a single isolated strain can take between two to three weeks, which is highly inconvenient when monitoring a control point in a food production process.

- Molecular biological methods.

Several methods for detecting toxigenic fungi based on molecular biology are currently being investigated. However, the most approximate methods are those based on RTPCR (real time PCR) that have a number of limitations when applied at an industrial scale. Firstly, few specific genes involved in the production of mycotoxins have been established, as for example the *Fusarium* genes *Tri4, Tri5* and *Tri6* for detecting species producing Group A and Group B trichotecenes. Moreover, although genes associated with other species producing aflatoxins and sterigmatocystin (*nor-1, ver-1, aflR, omt-A,* etc.) are useful for discerning these species from other fungi, they are not highly specific and it is not clear whether they can differentiate mycotoxigenic strains from non-producing strains within a particular species, as for example *Aspergillus flavus*. Another disadvantage of using real time PCR for detecting toxigenic fungi is that it is only capable of detecting the fungi when it is actively producing toxins, limiting the use of this test when the fungi are inactive or growing under conditions that are unfavourable for producing mycotoxins.



Aspergillus flavus

- Notwithstanding, the future of detecting and quantifying micotoxigenic fungi is based on **microarray technology (biochips)**. This technique allows one to perform comparative and simultaneous analyses of hundreds of genes in a device similar to a slide, in a very short period of time. However, many studies have yet to be undertaken to include species-specific gene fragments and sequences with phylogenetic information on the potential mycotoxin-producing species, together with essential genes for mycotoxin biosynthesis.[5,6] The previous description not only shows that establishing a monitoring system at a control point by trying to determine toxigenic fungi is highly complex and time-consuming, but also evidences the limitations of the methods used for detecting whether the fungi is producing the mycotoxins we are trying to control. Hence, the aforementioned methods are very useful for establishing studies regarding the existence and production of mycotoxins by fungi in certain foods and environments,

which would allow one to perform a hazard analysis, but are not applicable in the monitoring HACCP system once it is implemented.
For this reason, methods that analyze the mycotoxins present along the production chain of food products are chosen for monitoring control points. The analytical method selected depends on a number of factors such as the type of sample, the levels of mycotoxins to be detected, the control point to be monitored and the availability of technological, economical and human resources to perform the determination.[7]
The fastest methods used in the food industry are those based on ELISA technology using wells that provide quantitative results of many products at very convenient costs. It is important to take into account that the individual test kit must be validated for the mycotoxin and commodity in question. Therefore, when choosing a determined ELISA test kit it is important to confirm whether it is applicable to the product to be analyzed. Romer Labs® has performed over 80 validations for its ELISA AgraQuant® kits in different matrices. Many times, these types of determination, which take between 15 and 25 minutes, are used by industries for monitoring all the control points, even those requiring an analytical reference system.[8,9]
There are other commercially available methods for detecting mycotoxins that provide even faster determination, which are very useful especially at the reception point of raw materials. These are also immunological methods, like ELISA, but the test is performed using a strip type format which visually indicates the presence or absence of a particular mycotoxin using a reference cut-off point. Romer Labs® has the AgraStrip® series of lateral flow devices (LFDs) for determining aflatoxins and deoxynivalenol (DON) in different products such as corn, raisins, soy, nuts, wheat, etc. Three cut-off levels are available for aflatoxins: 4 ppb, 10 ppb and 20 ppb. This allows one to choose the most adequate strip test according to the commodity and its end use. For example, the AgraStrip® Afla 20 ppb is more convenient when analyzing corn to produce corn flour if the product will be commercialized in countries such as the US or the MERCOSUR region; however, the AgraStrip® Afla 4 ppb would be chosen when analyzing almonds to be exported to the European Community. Romer Labs® also offers an optical reader for its LFDs, the AgraStrip® **XR***eader*, which provides instant (semi-)quantitative results. As in the ELISA tests, it is necessary to verify that the analyzed matrix is included among the validated commodities.[10,11]

The use of biosensors is being evaluated for cases of liquid products such as wine, milk, beer, etc., which will have the advantage of providing online results, ensuring permanent control point monitoring. At the moment the developed systems require the extraction of the mycotoxin from the substrate with a solvent, but methods that can use the liquid matrix itself are currently under investigation.[12,13]

Confirmation and certification of results

The quality management plan must contemplate the correct functioning of the methods used for monitoring. There are two ways of ensuring this, which rather than being used exclusively, complement each other, and it is thus convenient to use both options to verify that the procedures provide precise and exact results.
The first option is using matrix reference materials (MRMs) or internal control samples. These are product samples used to audit the analytical procedure as they contain a known level of contamination with the mycotoxin of interest and their certificates include the value of uncertainty of determining the mycotoxin in the product. This reference or control sample is tested in the analytical system used, at a frequency established according to the number of analyses performed at that point. Romer Labs® subsidiary Biopure offers a wide range of MRMs for several toxins, and even with different levels of toxin contamination; these samples include a complete certificate of analysis which complies with ISO guides 31 & 35.[14]
The other option is to send samples from the different monitoring points to (reference) laboratories with analytical systems that can certify the value of the samples. This is also performed when wanting to confirm results that are very close to the acceptance or rejection point of the critical limit established. These laboratories use chromatographic techniques, HPLC, TLC, LC-MS/MS, GC-ECD, which are highly sensitive and exact. If possible, the laboratory must also be a certified or accredited entity (e.g. ISO 17025) or have a good Quality Assurance Plan to guarantee that their results are accurate, independently of the equipment or professionals they employ.[15,16] Romer Labs® has full service laboratories on three continents, in Singapore, the USA and Austria, all of which are certified (or are in process of certification) under the international norm ISO 17025 and have the most advanced technology, including LC-MS/MS.

Monitoring mycotoxins throughout the production chain is one safety measure. Other tools to prevent a contamination include the monitoring of environmental conditions that might favour the production of mycotoxigenic fungi. This can be done through measuring the water activity and the temperature in exposed areas.
A good HACCP system has to be capable of coping with all factors that put the production chain at risk. Whilst saying this, it is necessary to be aware that in this article only mycotoxins as one safety risk have been discussed.